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Docket No. VIP0007USA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Johan LENNERSTRAND, and Brendan LARDER
Serial No.	:	09/599,877
Filed	:	June 23, 2000
Title	:	Method for determining the mechanism of HIV RT inhibitors
Art Unit	:	1648
Examiner	:	Jeffrey S. PARKIN
Confirmation No.:		1424

DECLARATION

I, Dirk Edward Désiré JOCHMANS, resident at Grote Spekstraat 21, B-3020 Herent, Belgium, make the following declaration:

1. I graduated from the Catholic University of Leuven, Belgium, in 1993. In partial fulfillment of the graduation requirements, I submitted then a thesis entitled *Effect of Zinc on the Human Interferon-gamma Receptor*.
2. I earned a PhD in Sciences in 1998 from the same University. In partial fulfillment of the PhD requirements, I submitted then a thesis entitled *Over the aminoacid metabolism of Interferon-gamma stimulated cells*, and took courses on gene technology (Prof. G. Volckaert, Catholic University, Leuven) and signal transduction (Prof. M. De Ley, Catholic University, Leuven).
3. I have performed chemistry/biochemistry research work at the laboratory of Prof. Dr. M. De Ley, Laboratory of Biochemistry / Catholic University, Leuven) in collaboration with the Laboratory of Dr. J. Wietzerbin, Interferons and Cytokines Unit, INSERM / Institute Curie, Paris.
4. Part of my PhD work was developed in the following fileds:
   Cell culture: Isolation and purification of monocytes from human blood; non-specific esterase colouring;
   Stationary cultures: A549, COlo 205, HL-60, U-937, THP-1, K562, L929;
   Viral tests: EMCV/A549, VSV/WISH; Antibodies production in MiniPerm (Roller Bottle);

Transfection of human cell lines: Gene Pulser; X-Gal colouring; Culturing of human cells lines and yeasts; Colouring with trypan blue; MTT colouring; NBB colouring;

Immunocytochemistry colouring: Vectastain ABC; Preparation of cells for FACS; Scatchard analysis;

Protein analysis: Production, purification and marking (125-Iodine, biotin) of antibodies; SDS-PAGE and Western blotting; Immunologic colouring; Chromatography (FPLC); ELISA; EMSA (gel delay assay);

Molecular biology: RNA extraction; RT&Competitive PCR (Mimic); Construction of plasmids; Heterologous protein expression; Southern blotting; In vitro transcription/translation (TnT Promega); Screening of cDNA banks (Two Hybrid System); DNA sequence determination;

DNA analysis software: Clustal W, http://www.ebui.ac.uk; and

Other: Thin-layer chromatography; Aminoacid analysis.

5. I was a graduate assistant at the Department of Chemistry, Catholic University, Leuven, Belgium, form 1994-98. I am at present, and have been since 1998, a senior scientist at the Departmetn of Clinical Virology, Tibotec, Mechelen, Belgium.

6. I am the author or co-author of several publications in the chemical/biochemical field, including *Glutamine Synthesis in Human Monocytes*, D. Jochmans and M. De Ley (1998), Archives of Physiology and Biochemistry 106, B16, and WO03049746, *Combination of cytochrome P450 inhibitors and HIV protease inhibitors to treat retrovirus infections*.

7. I have read and understood the instant application.

8. The methods claimed in the instant application permit the determination of resistance levels that are higher than the levels that conventional methodologies can determine. The measurement of higher resistance levels in the claimed methodology is linked to the use of a ribonucleotide (feature of the claim "at least one ribonucleotide chosen from ATP and GTP or at least one pyrophosphate"). In the absence of a ribonucleotide, and in the presence of one HIV RT inhibitor, a RT enzyme will stop polymerizing when

contacting with the first RT inhibitor (which acts as terminator of the polymerization). In the presence of a ribonucleotide, pyphosphorolysis events occur, that means, that particular ribonucleotides are able to excise the RT inhibitors or terminator agents (such as AZT, 3TC, ddC, ddI, dideoxyATP) out from the polymerized piece of DNA, thus allowing further polymerization. Assays in the absence of ribonucleotides commonly measure 1 to 3 fold resistance. In the presence of ribonucleotides according to the claimed methodologies, up to 20-fold resistance is measured, as the RT enzyme becomes more resistant (can continue doing his job -polymerising- without being stopped).

9. Performing multiple chain termination events in a single well as in the presently claimed methodologies involves such a degree of complexity that its performance does not follow from conventional methodologies. Furthermore, and in contrast with the presently claimed methodologies, the measurement of multiple chain termination events in different wells would be very cumbersome, or even not suitable at all, for its implementation in a high throughput platform. In order to measure all events in one well, one needs to employ 4 differently labeled probes, in this case, 4 differently labeled dNTPs, e.g. 1 dNTP which is radioactive, a second dNTP which is luminescent, a 3rd dNTP which is fluorescent, and the 4th one which is detectable by absorption spectrometry. The claimed methodologies are very well suited for a high throughput platform.

10. Performing a plurality of resistance testing for RTS reactions in a single well does not follow from conventional methodologies. Commonly, without the use of ribonucleotides, one can only measure the stop of polymerization caused by a RT inhibitor for one base only (A, T, C, or G). In the presently claimed methodologies, one can measure the stop at A, T, C and G, thus one can perform and measure a plurality of reactions, because the presently claimed methodologies employ ribonucleotides, whose role is to excise the different RT inhibitors out from the DNA.

11. The undersigned is not aware, absent the teachings provided by the instant application, of conventional methodologies or references that, whether alone or in combination, would lead one to the development and implementation of the presently claimed methodologies. This is particularly so in light of what appears to be conflicting reports in this field. For example, Meyer, *et al.*, (1999),[1] report that their "results do not agree with those of Arion et al. (1998)",[2] and further specify that they "do not understand the reason for this difference." Meyer, *et al.*, p. 41, left col. (1999).

12. I declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and such willful statements may jeopardize the validity of the application or any patent issuing thereon.

Signed, this 16 day of September, 2003.

Dirk Edward Désiré JOCHMANS, PhD

[1] "A mechanism of AZTresistance: An increase in oligonucleotide-dependent primer unblocking by mutant HIV-1 reverse ttranscriptase", Mol. Cell. 4:35-43 (1999).

[2] ""Phenotypic mechanism of HIV-1 resistance to 3'-Azido-3'-deoxythymidine (AZT): Increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase", Biochem. 37:15908-15917 (1998).